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Serological and other biological differences among diadromous and lacustrine *Galaxias maculatus*-like forms from Chile (Pisces: Galaxiidae)*

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Abstract. The diadromous and the lacustrine *Galaxias maculatus* (or *G. maculatus*-like) populations of the Río Valdivia basin have striking differences in the electrophoretic pattern of their blood albumin. This and other biological findings suggest that they function as genetically separated units although they live in the same hydrographic system. This is a hint that they may belong to different species. If this is true, the available name for the lacustrine one would be *G. alpinus*. Nevertheless we do not revalidate it here once again, because we are waiting for more evidence from our ongoing immuno-serological research.

Key words. Pisces, Galaxiidae, *Galaxias maculatus*, Chile, blood serum electrophoresis, taxonomy.

Review of recent research

In 1972 McDowall gave a review of the *Galaxias maculatus* or *Galaxias maculatus*-like forms of the world. He considered them all conspecific. The oldest available name is *Mesites maculatus* given by Jenyns (1842) to the form from South America (Hardy Peninsula Tierra del Fuego). Since the oldest name for the genus is *Galaxias* Cuvier 1817, the valid combination is *Galaxias maculatus*.

A synonymy list was given by McDowall (1971): 49; 1972: 335) discussing taxonomical problems (See also Stokell 1966; McDowall 1967, 1970, 1981). Based on extensive morphometric studies he showed that they were all very similar and concluded that they belong to a single species widespread on the southern continents. They gave rise to diadromous populations with estuarine or sea dwelling larval stages and lacustrine ones which fulfill their whole life-cycle in freshwater. The most distinct character was the lower number of vertebrae of the lacustrine populations (McDowall op. cit.). He considered this character not a genetical fixed one but due to the direct influence of different conditions of the environment during the embryonal development (McDowall 1971: 55; 1972: 352).

In 1973 one of the authors (Campos) in a study on migrations of *Galaxias maculatus* in Valdivia estuary remarked again the differences between the amount of vertebrae in the estuarine populations and the ones of some lakes from Chile; he showed also a bimodal curve in the vertebrae counts in the migratory populations in the Valdivia river estuary, but without inferring a taxonomical consequence. He only compared the similar situation found by McDowall (1968) in New Zealand populations and supported the view of McDowall (1967—1981) and Stokell (1966) that admits one species with diadromous and lacustrine populations throughout the different continents.

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It was in 1974 that Campos in a study of the *G. maculatus* of the Rio Valdivia basin postulated that the taxon known under this name in Chile must consist of two different genetically isolated units: so he assigned them to different species. He revalidated *G. alpinus* Valenciennes 1846. He based this statement on the existence of the discontinuity of the number of vertebrae linked with other morphological features. Other interesting conditions he found were that in pre-adult stages they mix with one another segregating at the adult or spawning stages. Also the change from juvenile to adult in *G. maculatus* is more drastic than in *G. "alpinus"*. He assumed that the differences were governed genetically. So it should be for behavioural, physiological and other differences.

In 1976 McDowall published a reply to Campos' work in which he doubted about Campos' hypothesis. He argued that the difference in vertebrae numbers, migration behaviour etc. were not enough to sustain the assumption of two species. He said that Campos had not tried enough to understand the dynamics of variation in various widespread populations, nor had he adequately considered the problem of reproductive isolation.

A very strong argument of McDowall was that there is no reason to assume in nearly each case of lacustrine populations will be selective pressures leading to a decline of vertebrae numbers, a condition that supports the point of view that these changes are directly induced by modificatory environmental factors upon the embryos and may not be due to genetical differences. Indeed, a very controverse but interesting problem. Recently one of the authors (Busse) had occasion to visit Chile and to discuss the problem with the other one (Campos) at the Instituto de Zoología in Valdivia. We arranged to collaborate in search of a solution by means of biochemical methods.

Material and Methods

a) Sampling

Diadromous and lacustrine samples of *Galaxias maculatus* and "*G. alpinus*" populations were caught in Río Cau-Cau (Valdivia) and Lago Riñihue respectively. Their blood was extracted by heart punction (fig. 1) and afterwards centrifugated. The serum was deep-frozen in approx. 100 μl portions. (For this amount 20 ore more specimens were necessary).

The samples were analysed by polyacrylamid-electrophoresis by the same method used by Joger 1984 (details see on b). On each plate could be applied 6 different serum samples. We applied 2 to 5 μl of serum per sample.

b) Polyacrylamid Electrophoresis

We used an electrophoresis system similar to the method described by Taggart et al. (1978). The apparatus was GE — 2/4 LS Pharmacia (Sweden) in which 4 electrophoresis slabs can be introduced vertically. The system is cooled by water.

The stock solutions were prepared to be stored up to three weeks and in adequate quantities for the electrophorese apparatus as follows.

Solution I:
40 g Acrylamide (Ferak 81853)
1 g Bis (= N,N' Methylenbisacrylamide 2x crist.; Serva 29195)
H_2O dest. (dissolved warm and filled up to 200 ml).

Solution II:
18,17 g Tris (= Hydroxymethylaminomethan $C_4H_{11}NO_3$; Merck 8382)

dissolved in 30—40 ml H_2O (warm) with conc. HCl adjusted to pH 8,8
H_2O (filled up to 100 ml).

Solution III:
6,06 g Tris (as above but adjusted to pH 6,8).

Solution IV: Buffer
6,0 g Tris
28,89 g Glycin (NH_2CH_2COOH; Ferak 00752)
H_2O (filled up to 1000 ml), pH 8,7, dissolved to 1/5 for use.

Solution V:
100 mg AP (= Ammoniumperoxdisulfate $(NH_4)_2S_2O_8$; Ferak 00177)
H_2O (dissolved in 5 ml; prepared directly before use).

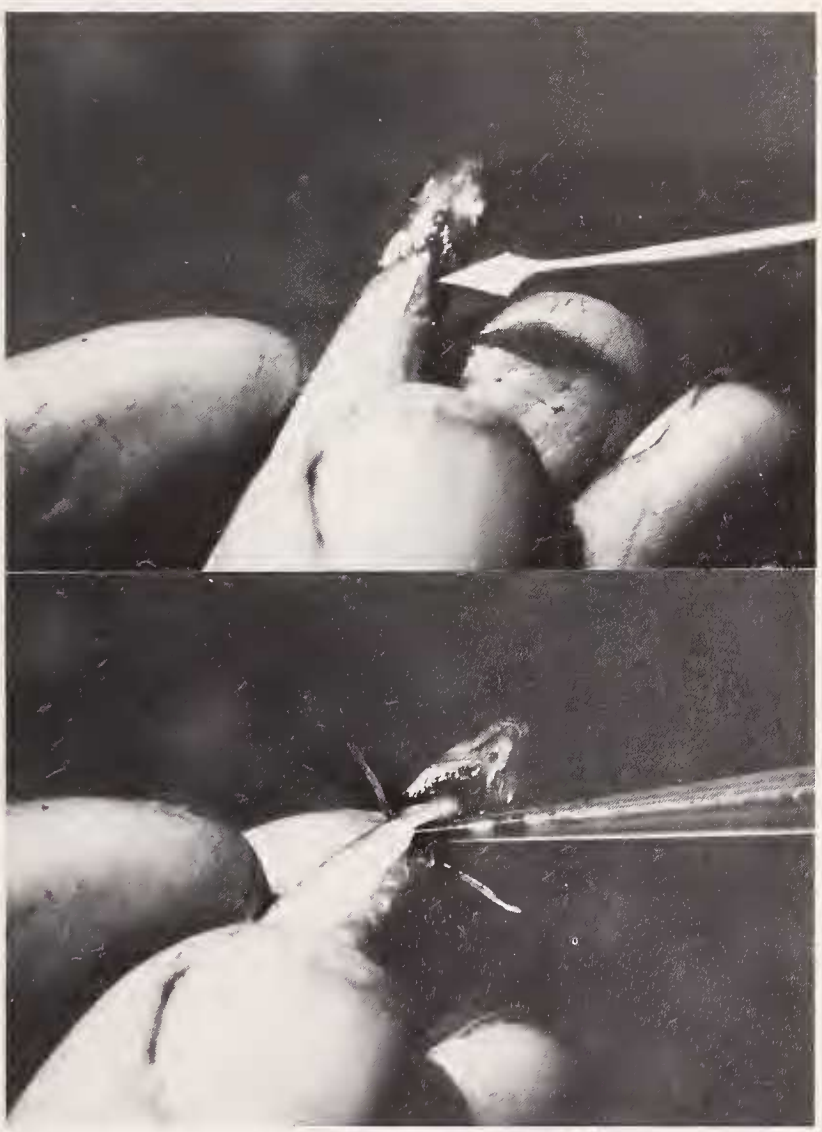

Fig. 1: Taking blood from *Galaxias* by means of heart punction and extraction with a pipette. The heart is behind the isthmus. In the living animal a pulsating rhombical area of the silvery skin indicates the real location. On the photograph a specimen belonging to the lacustrine population of Lago Riñihue.

Preparing of the gel slabs:

Stacking gel	Separation gel
Sol. III 6 ml	Sol. II 21 ml
„ I 3,6 ml	„ I 31,5 ml
„ V 0,3 ml	„ V 1,8 ml
H_2O 14,1 ml	H_2O 29,7 ml
— Degasing —	— Degasing —
T-med 15 µl	T-med 30 µl

These quantities are necessary for 4 slabs to be prepared in the gel slab casting apparatus (for 8 slabs the double amount). T-med is added after degasing and immediately before casting the geles. The first one is the stacking gel. It is covered with a thin layer of isobutanol which is washed away after solidification; then the separating gel is applied. The slabs are ready for use after some hours or the next day.

Applying of samples and run of electrophoresis:
The samples are applied when the whole apparatus is mounted with the gel slabs and the buffer solution. For this purpose 2—5 µl of serum were mixed with 5 to 15 µl of concentrated sugar solution slightly stained with brome phenole blue.

The electrophoreses were run the first 15 min. at 50 V, the next 30 min. at 75 V and the following approx. 2½ hours at 110 V. They were stopped when the front was about 2 cm near the edge of the slab.

The staining method used was Coomasie blue.

c) Radiographs
To document that the serum we used came from no other forms than those under discussion we fixed the bleeded specimens in diluted alcohohl-formol and conserved them in 70 % alcohol. In order to count the vertebrae subsamples of both populations were radiographed (see fig. 3).

Results

a) Biochemical approach

The electropherogram of the blood sera of the two populations resulted quite different (fig. 2). The first band of albumin is very heavy in comparison to all others in the lacustrine form "*alpinus*"). The second one is almost absent in this form and if present nearer to the third, while in the diadromous *maculatus* they are all similar in strength and almost equidistant. Also there are differences in the bands of other proteins including the residual haemoglobin present in the serum by means of the unavoidable amount of haemolysis during the blood extraction and centrifugation.

b) Morphological approach

This is congruent with the findings known up to this day. To illustrate them, it seemed advisable to present radiographs (fig. 3).

Discussion

It is highly probable that the differences found in the albumins of blood are due to genetic differences. It could be argumented that these might be only individual ones. This is unprobable because each electropherogram was made upon a sample of at least 20 individuals. So they represent a mean of the population. Each of them has a clear characteristic not shared with the other, so that we can assume in contrast

Fig. 2: Electropherogrammes of the blood serum from three populations *of Galaxias maculatus* or *G. maculatus*-like forms. Left *G. maculatus** from Australia; middle *G. maculatus* from the estuarine population from Chile (Río Cau-Cau near Valdivia); right *G. maculatus** from the lacustrine population (Lago Riñihue, Río Valdivia system). * In this cases the name "*maculatus*" is used with reserve.

to McDowall (1972: 351) that there is no gene flow between both. The presence of such a clear discontinuity among two sympatric or at least parapatric populations is an argument to consider them as different species.

The differences in the other nonalbumin protein fractions of the blood like the haemoglobin (or transferrins) in general lead to the same conclusion. It must be mentioned nevertheless, that in other species like in *Salmo salar* or *Clupea harengus* (Koch, Bergström & Evans 1964; Wilkins & Iles 1966, respectively) there are haemoglobin differences linked with different demands in oxygen carrying capacity in different stages of growth or age. In our case the latter can be neglected because they were all of approximately the same size. It persists only a slight doubt by means of a possible modificatory action of the environment via these different demands on the haemoglobin. This may weaken this argument but is not necessarily an argument against the genetical distinctness.

In relation to this environmental response there is a striking physiological difference between the two populations. While the diadromous ones can be caught and held in a tank or in aquaria without problems, the lacustrine individuals usually die within half an hour after catching. It seems not only to be a different response to

oxygen availability in the water but a different stress physiology in general. Also here it is likely to postulate genetic differences. As Campos observed the two forms together in some parts of the same river system in nonreproductive stages, to explain the subsequent segregation it is necessary to postulate different behavioural programs for each to find the right spawning sites. Even though spawning traditions can be established by an imprinting process of the birth place water like in salmons, it is probable that such complex behavioural finding mechanisms have at least an inherited base. They are to be considered as another discontinuity reinforcing the assumption of different species. Otherwise in some closely related species, for instance in birds, it is common that they build up mixed swarms in their social phase and segregate in their reproductive phase (Busse 1975).

The morphological discontinuity like different numbers of vertebrae (also in the general habitus there are slight differences) may, but must not necessarily be the result of modificatory environmental conditions so that they are not the appropriate arguments to sustain conspecificy.

For all these reasons it seems more likely that *Galaxias maculatus* and *G. "alpinus"* are different species. But for this moment we do not revalidate the name *"alpinus"* Valenciennes once again, because we are waiting for a higher degree of certainty expected from further investigation in immunology and zoogeography, respectively.

To obtain an appreciation of the degree of divergence in serum proteins we tried to compare both forms by means of immunological methods. For technical reasons

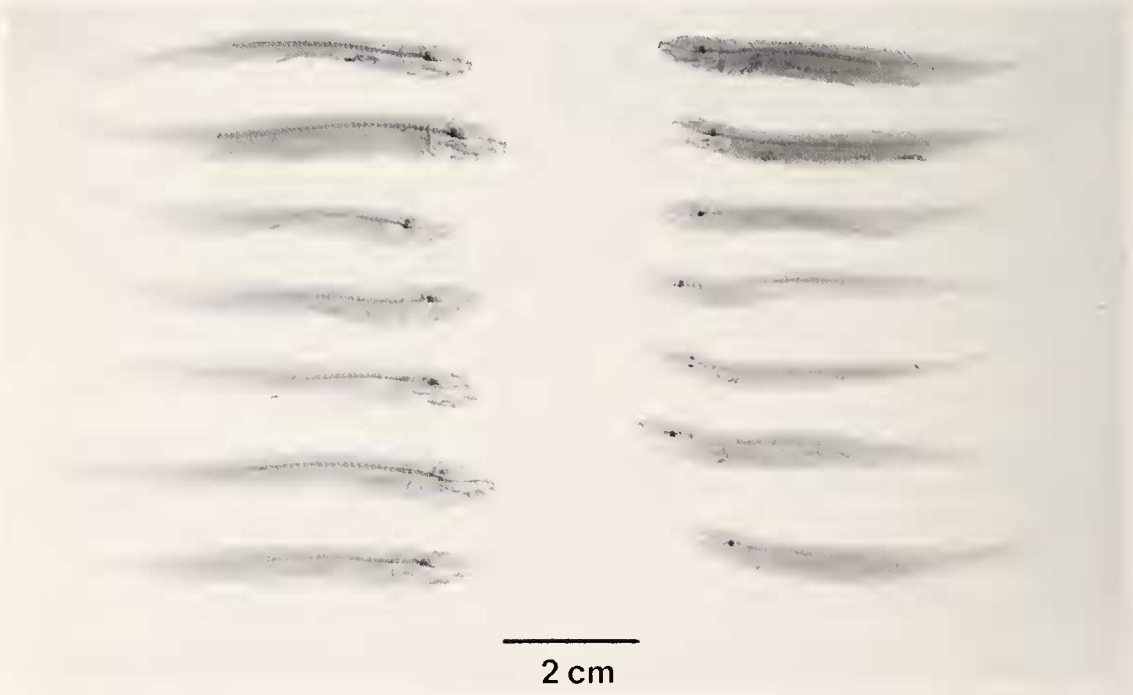

Fig. 3: Radiographs of estuarine (left) and lacustrine (right) *Galaxias maculatus* or *G. maculatus* like forms from Chile. The estuarine ones from Lago Riñihue, a lake of the Río Valdivia system.

and the small amount of proteins obtained from these tiny fishes, we have still no suitable results, but we hope refined methods will apply for this purpose.
The other reason why we do not pronounce in favour of the specificity of these two forms is that similar conditions could be expected in all other *Galaxias maculatus* (or *G. maculatus*-like) populations from Australia and New Zealand and it would be advisable to know more about the whole group before drawing taxonomic conclusions.

Zusammenfassung

Die beiden in dem Einzugsgebiet des chilenischen Flusses Río Valdivia lebenden Formen von Galaxiiden, die zur Zeit der Art *Galaxias maculatus* zugerechnet werden, weisen erhebliche Unterschiede im Elektropherogramm ihres Blutserums auf. Während die kathadrome in den Flußmündungen laichende Population drei mittelmäßig starke „Albumin"banden aufweist, hat die andere — sich in den Seen fortpflanzende — eine sehr viel stärkere erste Bande und zwei schwächere, wovon die mittlere und schwächste an etwas anderer Stelle als die entsprechende der Flußmündungsform steht. Diese Unterschiede, sowie auch solche der Entwicklung, der Körpergestalt, der Physiologie und des Verhaltens zeigen eine Diskontinuität auf, die besagt, daß die beiden Populationen unterschiedlichen Fortpflanzungsgemeinschaften angehören dürften. Dieses könnte einen unterschiedlichen Artstatus rechtfertigen. Sofern diese Hypothese zutrifft, würde der für die Seenform verfügbare Name „*Galaxias alpinus*" lauten. Dieser wird jedoch an dieser Stelle nicht revalidiert, da wir zu diesem Schritt einen höheren Grad an Sicherheit abwarten. Laufende immunserologische Untersuchungen haben aufgrund der sehr geringen Mengen der von diesen kleinen Fischen gewonnenen Proteine noch keine Ergebnisse erbracht. Aber wir erwarten von verfeinertern Methoden Aufschlüsse zur genaueren Klärung des Verwandtschaftsgrades beider Formen.

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